"The United States government has rights in the present invention pursuant to grant number NS29225 from the National Institute of Neurological Disorders and Stroke."

In The Claims

Please cancel claims 1 and 6-11 without prejudice.

Please cancel claims 2-5 and 12-21 without prejudice.

Please add the following new claims 22-53:

22. A microclonal cDNA library representing a collection of expressed gene transcripts produced from a single microclone, wherein said microclone comprises a population of cells in which each cell is the progeny of a single clonogenic stem/progenitor cell.

23. The microclonal cDNA library of claim 22 wherein the stem/progenitor cell is a neural stem/progenitor cell.

24. The microclonal cDNA library of claim 22 wherein the stem/progenitor cell is a tumor cell.

25. The microclonal cDNA library of claim 22 wherein the tumor cell is a glioma cell.

26. The microclonal cDNA library of claim 22 wherein the stem/progenitor cell is a hematopoietic cell.

27. The microclonal cDNA library of claim 23 wherein the microclone is a neurosphere cultured from a brain.

28. The microclonal cDNA library of claim 27 wherein the brain is from a human.

29. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce an early type I neurosphere.

30. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce a late type I neurosphere.

31. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce a type II neurosphere.

32. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce a small neurosphere.

33. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce a medium neurosphere.

34. The microclonal cDNA library of claim 27 wherein the clonogenic stem/progenitor cell is cultivated under conditions to produce a large neurosphere.

35. A collection of developmentally sequential microclonal cDNA libraries, said collection prepared by the following steps:

(a) cultivating a stem/progenitor cell to produce a microclone;

(b) disrupting said microclone and amplifying RNA of cells comprising said microclone;

(c) preparing a cDNA library from said amplified RNA;

(d) preparing a plurality of cDNA libraries according to steps (a)-(c) from developmentally diverse stem/progenitor cells;

(e) compiling a gene expression profile for each said microclonal cDNA library by analyzing the cDNAs of each said library for the presence of transcripts for at least one selected marker of development or cell phenotype;

(f) comparing the expression of at least one said selected marker in at least two said microclonal cDNA libraries; and

(g) characterizing the developmental stage of said microclonal cDNA libraries relative to a developmental sequence based on the expression profile of the markers in each cDNA library.

36. The collection of developmentally sequential microclonal cDNA libraries of claim 35 wherein the microclone is a neurosphere.

37. The collection of developmentally sequential microclonal cDNA libraries of claim 36 wherein the marker of step (e) is selected from the group consisting of β-actin, β-2 microglobulin, neuron-specific enolase, neurofilament-M, MAP-2, PAX-6, tenascin, nestin and GFAP.

38. A subtractive cDNA library representing gene transcripts that are differentially expressed at a selected stage of development in a developmental sequence wherein the library is prepared by a method comprising the steps of:

(a) cultivating a clonogenic stem/progenitor cell to produce a first microclone comprising cells at a first stage of development;

(b) disrupting said microclone and amplifying RNA of said cells of said first microclone;

(c) preparing a first microclonal cDNA library from said amplified RNA;

(d) preparing a second microclonal cDNA library from RNA of cells of a second microclone at a second stage of development;

(e) comparing RNA transcripts from said first and said second cDNA library by subtractive hybridization to identify a set of differentially expressed transcripts associated with said first but not said second cDNA library; and

(f) correlating expression of said identified transcripts with said selected stage of development.

39. The subtractive cDNA library of claim 38 wherein the developmental sequence is neuromorphogenesis.

40. The subtractive cDNA library of claim 38 wherein the developmental sequence is oncogenesis.

41. The subtractive cDNA library of claim 38 wherein the developmental sequence is hematopoeisis.

42. The subtractive cDNA library of claim 38 wherein said first microclone and said second microclone is a neurosphere.

43. The subtractive cDNA library of claim 42 wherein the first neurosphere is an early type I neurosphere and the second neurosphere is a late type II neurosphere.

44. The subtractive cDNA library of claim 42 wherein the first neurosphere is an early type I neurosphere and the second neurosphere is a late type I neurosphere.

45. The subtractive cDNA library of claim 42 wherein the first neurosphere is a late type I neurosphere and the second neurosphere is a type II neurosphere.

46. The subtractive cDNA library of claim 42 wherein the first neurosphere is small neurosphere and the second neurosphere is a large neurosphere.

47. The subtractive cDNA library of claim 42 wherein the first neurosphere is a small neurosphere and the second neurosphere is a medium neurosphere.

48. The subtractive cDNA library of claim 42 wherein the first neurosphere is medium neurosphere and the second neurosphere is a large neurosphere.

49. The subtractive cDNA library of claim 38 wherein the first cDNA library and the second cDNA library are a neighboring pair in a collection of developmentally sequential microclonal cDNA libraries prepared according to claim 35.

50. The subtractive cDNA library of claim 39 comprising transcripts associated with early development of pluripotent stem cells, said transcripts being obtained by subtractive hybridization of a first and a second microclonal cDNA library prepared from a type I neurosphere, said first and second cDNA libraries being characterized by absence of transcripts for Pax-6.

51. The subtractive cDNA library of claim 39 comprising transcripts associated with early development of pluripotent stem cells, said transcripts being obtained by subtractive hybridization of a first and a second microclonal cDNA library prepared from a small neurosphere, said first and second cDNA libraries being characterized by absence of transcripts for Pax-6.

52. A DNA array comprising DNA fragments identified in a subtractive cDNA library prepared according to claim 38.

53. The microclonal cDNA library of claim 28 wherein the brain is from a human with a disease selected from the group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease and brain cancer.

REMARKS

Status of The Claims

Claims 1 and 6-11 have been cancelled without prejudice and without disclaimer. The election of originally filed claims 2-5 and 12-18 is made without traverse.